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Rapid tolerance to the hypotensive effects of glyceryl trinitrate in the rat: prevention by N-acetyl-L- but not N-acetyl-D-cysteine

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1 A new model of tolerance to the hypotensive effect of organic nitrates has been developed in the rat.

2 The fall in mean arterial pressure (MAP) in response to bolus doses of sodium nitroprusside (NP) (4 $\mu g\,kg^{-1}$) and glyceryl trinitrate (GTN) (10 $\mu g\,kg^{-1}$) was recorded both before and after a 60 min infusion of either 0.9% saline, NP (20 $\mu g\,kg^{-1}\,min^{-1}$) or GTN (40 $\mu g\,kg^{-1}\,min^{-1}$).

3 The hypotensive effects of NP or GTN were unchanged following saline infusion, but were reduced in both cases by approximately 40% following the infusion of NP.

4 Infusion of GTN for 60 min virtually abolished the hypotensive effect of a GTN bolus (i.e. nitrate tolerance), whilst the effect of a NP bolus was reduced only to a similar extent (30%) as after an infusion of NP. This latter effect is attributed to a degree of non-specific cross-tolerance between GTN and NP.

5 Co-treatment of a group of rats with N-acetyl-L-cysteine (L-NAC) prevented the development of nitrate tolerance, confirming the role of thiols in this phenomenon, whereas N-acetyl-D-cysteine (D-NAC) did not.

6 The stereospecificity in the effect of NAC in preventing this specific tolerance to GTN suggests that the interaction between GTN and NAC and/or cysteine involves an enzyme-dependent step.

7 NAC was unable to prevent the non-specific cross-tolerance to NP which followed infusion of GTN, suggesting that the mechanism does not directly involve NAC and/or cysteine.

Introduction

Organic nitrates (such as glyceryl trinitrate, GTN), nitric oxide (NO) and NO-containing nitrocompounds (such as sodium nitroprusside, NP) exert their vasodilator action through the activation of soluble guanylate cyclase (Ignarro *et al.*, 1984). There is evidence for the involvement of intracellular thiols at one or more sites within the biochemical pathway that culminates in the activation of this enzyme. Firstly, stimulation of partially purified soluble guanylate cyclase by NO or NO-containing nitrocompounds *in vitro* requires the presence of thiols, probably as precursors for the formation of S-nitrosothiol intermediates (Ignarro *et al.*, 1981). Secondly, thiols may be involved at a stage in the biotransformation of organic nitrates which precedes the formation of NO or S-nitrosothiol intermediates, though the exact mechanism remains a subject of some controversy (Needleman *et al.*, 1973; Ignarro *et al.*, 1981; Yeates *et al.*, 1985).

Tolerance to the beneficial effects of organic nitrates can develop within 24 h of continuous administration (Elkayam *et al.*, 1987). Needleman & Johnson (1973) demonstrated that the induction of tolerance to GTN *in vivo* in the rat was accompanied by depletion of tissue sulphydryl groups, and several clinical studies have shown prevention and reversal of nitrate tolerance by the cysteine precursor N-acetylcysteine (Packer *et al.*, 1987; May *et al.*, 1987). These data are compatible with the hypothesis that it is the unavailability of critical intracellular sulphydryl groups, possibly in the form of cysteine, which determines the development of nitrate tolerance. The results of other studies performed *in vitro*, however, suggest that more than one mechanism may be involved (Gruetter & Lemke, 1985; Abdollah *et al.*, 1987; Mulsch *et al.*, 1988; Henry *et al.*, 1989; Romanin & Kukowetz, 1989). In an attempt to elucidate further the mechanism by which N-acetyl-cysteine (NAC) prevents tolerance *in vivo*, we have developed a new model of tolerance to the hypotensive effects of intravenous GTN in the anaesthetized rat. We have used this model to study the interaction between GTN and the two enantiomers of NAC, and to compare the effects of GTN with those of an inorganic nitrate, NP.

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Methods

Preparation of animals

Male Sprague-Dawley rats (200–450 g) (obtained from Harlan Olac Ltd, Bicester, U.K.) were anaesthetized with sodium pentobarbitone (60 $mg\,kg^{-1}$ i.p. and 40 $mg\,kg^{-1}$, s.c.) and placed on a heated mat (37–39°C, Remploy). Further anaesthetic (sodium pentobarbitone 40 $mg\,kg^{-1}$, i.p.) was administered 20 min after the start of the 60 min infusion period. The trachea was cannulated and blood pressure recordings were made via a polyethylene cannula inserted into the right carotid artery, using a Gould pressure transducer (model P321D) and Grass polygraph recorder (model 79D). Intravenous drugs were administered via a polyethylene cannula inserted into the right jugular vein. Heparin (1 iu g^{-1}, i.v.) was administered immediately after cannulation, and the experiment was begun after a 10 min equilibration period.

Experimental protocol

Two bolus doses of NP (4 $\mu g\,kg^{-1}$) and then of GTN (10 $\mu g\,kg^{-1}$) were administered i.v. both before and again 5 min after a 60 min infusion of either 0.9% saline ($n = 4$), NP (20 $\mu g\,kg^{-1}\,min^{-1}$) ($n = 8$) or GTN (40 $\mu g\,kg^{-1}\,min^{-1}$) ($n = 8$). Two additional groups of animals received either L-NAC ($n = 8$) or D-NAC ($n = 8$) before and during a 60 min infusion of GTN (40 $\mu g\,kg^{-1}\,min^{-1}$) as follows: 200 $mg\,kg^{-1}$ in 1 ml 0.9% saline i.p. and 200 $mg\,kg^{-1}$ in 0.5 ml 0.9% saline administered i.v. before infusion, and 200 $mg\,kg^{-1}$ (in 0.9% saline) at 5 $ml\,kg^{-1}\,h^{-1}$ over the 60 min infusion period. A 'Y' connector system ensured that extracorporeal admixture of GTN and NAC was kept to a minimum. In all experiments the total infusion volume was 10 $ml\,kg^{-1}$ over 60 min.

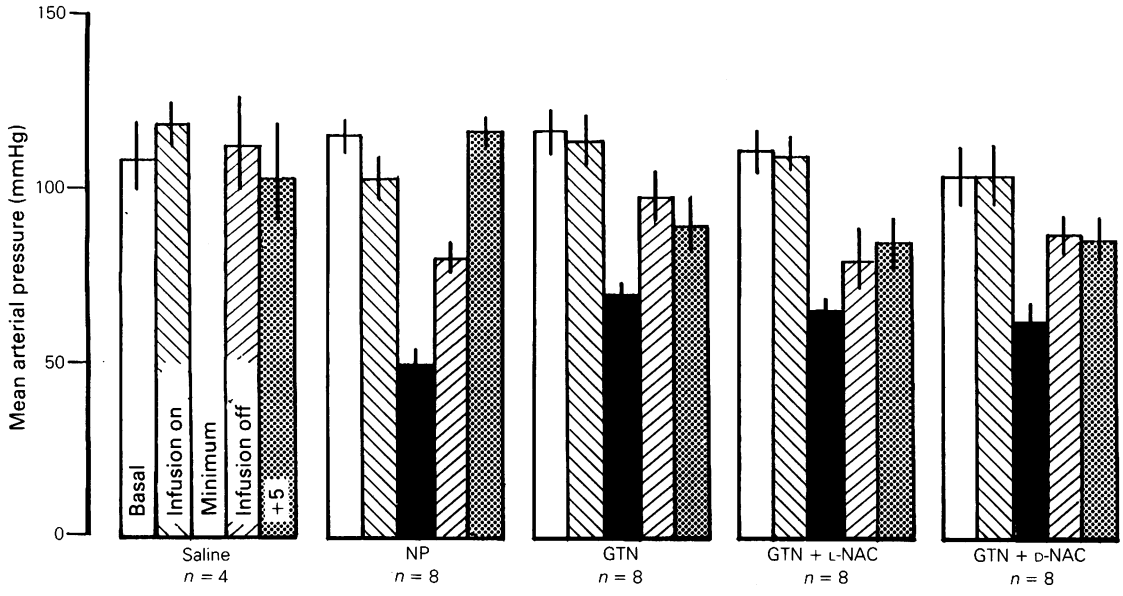

Figure 1 Mean arterial pressure (MAP) recorded at the onset of the experiment (Basal) and at the beginning (Infusion on), the end (Infusion off) and 5 min following (+5) a 60 min i.v. infusion of 0.9% saline, sodium nitroprusside (NP) ($20\,\mu g\,kg^{-1}\,min^{-1}$) or glyceryl trinitrate (GTN) ($40\,\mu g\,kg^{-1}\,min^{-1}$) in rats anaesthetized with sodium pentobarbitone ($60\,mg\,kg^{-1}$, i.p. and $40\,mg\,kg^{-1}$, s.c.). MAP fell rapidly during the infusion of NP and GTN, reached a minimum (Minimum, solid column) within 2 min of the onset, but then rose towards the pre-infusion level during the remainder of the infusion period. MAP did not decrease during infusion of 0.9% saline, so that no value for Minimum is shown for this group of animals. Two groups of animals were co-treated with either N-acetyl-L-cysteine (L-NAC) or N-acetyl-D-cysteine (D-NAC) (total dose $600\,mg\,kg^{-1}$) before and during the infusion of GTN (see text), but such treatment had no effect on basal MAP. Values are mean for numbers of animals indicated with s.e. shown by vertical lines.

Drugs

Glyceryl trinitrate (as a 0.5% solution in 30% v/v ethanol and 30% v/v propylene glycol) and sodium nitroprusside powder were obtained from David Bull Laboratories. N-Acetyl-L-cysteine and D-cystine were obtained from Sigma Chemical Co. N-Acetyl-D-cysteine was synthesized according to the method described by Sheffner *et al.* (1966). Essentially, D-cystine (2 g) was dissolved in aqueous sodium hydroxide (2 M, 8.7 ml) and cooled in an ice/salt bath. Six portions of acetic anhydride (0.7 ml) and sodium hydroxide solution (6.6 ml) were added and the solution allowed to stand for 0.5 h in the cold and a further 0.5 h at room temperature. Sodium ions were removed by passage through a Dowex column (H^+ form, 25 × 1 cm). Acetyl-D-cystine was reduced by zinc/acetic acid at 60°C for 3 h; zinc ions were removed on a Dowex (H^+) column and the resulting solution lyophilised. N-Acetyl-D-cysteine was recrystallised from hot water. The molecular rotation of the product was confirmed as that of the D-isomer by Miss S. Johnson, Department of Chemistry, Imperial College, London.

Analysis of data

Mean arterial pressure (MAP) was estimated from the systolic (SDP) and diastolic (DBP) blood pressure recordings using the formula MAP = DBP + ((SBP − DBP)/3). All results have been expressed as mean ± s.e.mean. Comparisons amongst groups were made using two-way analysis of variance, and individual comparisons using Student's *t* test for paired samples. The null hypothesis was rejected at $P < 0.05$.

Results

Basal MAP was similar in all groups of rats, including those co-treated with either L-NAC or D-NAC (Figure 1). Infusion of 0.9% saline for 1 h had no effect on MAP ($n = 4$). During infusion of GTN ($40\,\mu g\,kg^{-1}\,min^{-1}$), MAP fell rapidly to a minimum of 69.4 ± 3.5 mmHg, rose progressively over the remainder of the 60 min infusion period, but remained below the preinfusion MAP during the first 5 min after completion of the infusion. A similar pattern was observed in those animals co-treated with either L-NAC or D-NAC (Figure 1). Infusion of NP at a dose of $20\,\mu g\,kg^{-1}\,min^{-1}$ resulted in a somewhat greater fall in MAP, reaching a minimum of 49.4 ± 4.2 mmHg. MAP again rose progressively over the remainder of the infusion period, recovering rapidly during the immediate post-infusion period to reach pre-infusion MAP within 5 min (Figure 1).

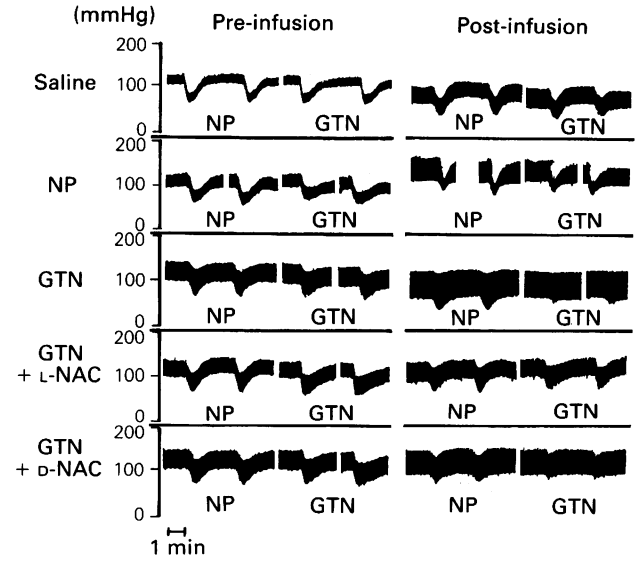

Figure 2 Representative individual arterial pressure recordings showing the hypotensive effect of duplicate intravenous bolus doses of sodium nitroprusside (NP) ($4\,\mu g\,kg^{-1}$) and glyceryl trinitrate (GTN) ($10\,\mu g\,kg^{-1}$) before (Pre-infusion) and 5 min after a 60 min infusion (Post-infusion) of 0.9% saline, NP ($20\,\mu g\,kg^{-1}\,min^{-1}$) or GTN ($40\,\mu g\,kg^{-1}\,min^{-1}$). GTN infusions were also administered to animals which had been co-treated with either N-acetyl-L-cysteine (L-NAC) or N-acetyl-D-cysteine (D-NAC) (total dose $600\,mg\,kg^{-1}$).

Table 1 Baseline and minimum (min.) mean arterial pressure (MAP) (±s.e.mean) in response to intravenous bolus doses of sodium nitroprusside (NP) (4 $\mu g\,kg^{-1}$) and glyceryl trinitrate (GTN) (10 $\mu g\,kg^{-1}$), both before and 5 min after the 60 min infusion period

	Pre-infusion				*Post-infusion*			
Infusion	*NP*		*GTN*		*NP*		*GTN*	
type	*Baseline*	*Min.*	*Baseline*	*Min.*	*Baseline*	*Min.*	*Baseline*	*Min.*
Saline	112.6	70.6	112.3	67.3	120.8	77.0	119.3	78.6
	(7.7)	(2.8)	(8.2)	(2.4)	(10.4)	(5.8)	(11.4)	(7.7)
NP	115.6	72.4	106.3	69.6	127.2	103.4	126.3	102.0
	(3.1)	(2.8)	(3.3)	(2.3)	(4.2)	(4.2)	(3.8)	(4.0)
GTN	120.6	76.5	119.7	82.0	110.6	80.5	116.1	106.6
	(5.8)	(3.8)	(4.8)	(3.2)	(3.8)	(4.3)	(4.0)	(4.3)
GTN +	113.3	75.3	109.2	76.8	96.5	73.1	100.0	79.8
L-NAC	(4.0)	(2.1)	(3.8)	(2.4)	(5.5)	(4.4)	(6.2)	(4.7)
GTN +	105.5	75.7	102.6	77.5	104.3	79.8	109.1	97.6
D-NAC	(5.1)	(4.4)	(4.4)	(4.0)	(2.8)	(2.8)	(2.9)	(3.4)

It was established during preliminary experiments that bolus injections of NP (4 $\mu g\,kg^{-1}$) and GTN (10 $\mu g\,kg^{-1}$) in untreated anaesthetized rats caused a similar, transient, fall in MAP, and these doses were used thereafter. Representative tracings from each group of animals are shown in Figure 2 and the pooled results in Table 1 and Figure 3. There was no significant difference between groups in the hypotensive effect of bolus doses of NP or GTN administered immediately after the equilibration period, including those animals which had been pretreated with either L-NAC or D-NAC. The fall in MAP induced by a bolus dose of NP was similar pre- and post-infusion of 0.9% saline for 60 min (42.0 ± 9.1 mmHg pre-, 43.8 ± 8.0 mmHg post-), but was significantly and similarly attenuated following infusion of NP (43.3 ± 2.5 mmHg pre-, 23.8 ± 2.5 mmHg post-, $P < 0.001$) and GTN (44.0 ± 4.6 mmHg pre-, 30.1 ± 3.7 mmHg post-, $P < 0.05$), most probably due to a degree of non-specific tolerance to NP. This reduction in the effect on MAP of a bolus dose of NP following a GTN infusion was unaffected by co-treatment with L-NAC (fall in MAP 38.0 ± 3.9 mmHg pre-, 23.4 ± 2.3 mmHg post-infusion of GTN, $P = 0.005$), although D-NAC seemed to have some protective effect (31.9 ± 3.5 mmHg pre-, 24.6 ± 2.8 mmHg post-, $P > 0.1$). However, this appeared to be due to the fact that the hypotensive response to the pre-infusion bolus dose of NP was impaired in these animals, the reason for which is not clear.

The hypotensive effect of a bolus dose of GTN was also unaffected by infusion of 0.9% saline for 60 min, and was attenuated following the infusion of NP (fall in MAP 36.7 ± 2.4 mmHg pre-, 24.3 ± 2.4 mmHg post-, $P < 0.005$) by a similar degree to that observed for the bolus dose of NP post-infusion of NP, again suggesting a degree of non-specific cross-tolerance between GTN and NP. Following infusion of GTN, however, the hypotensive effect of a bolus dose of GTN was markedly reduced (37.7 ± 4.1 mmHg pre-, 9.6 ± 1.1 mmHg post-, $P = 0.0001$), indicating an additional element of tolerance specific to GTN and not NP. The specific component of the tolerance to GTN could be completely prevented by co-treatment with L-NAC (fall in MAP 32.3 ± 2.8 mmHg pre-, 20.2 ± 2.7 mmHg post-) but not D-NAC (26.8 ± 2.5 mmHg pre-, 11.5 ± 1.9 mmHg post-), ($P = 0.001$ by ANOVA). This is perhaps most clearly illustrated by comparisons of the ratio of the fall in MAP induced by a bolus of GTN compared to that induced by NP (GTN : NP ratio) under different conditions (Figure 3).

Discussion

The mechanisms responsible for vascular tolerance to organic nitrates remain unclear. Previous work in rats has shown that tolerance induced *in vivo* is accompanied by depletion of tissue

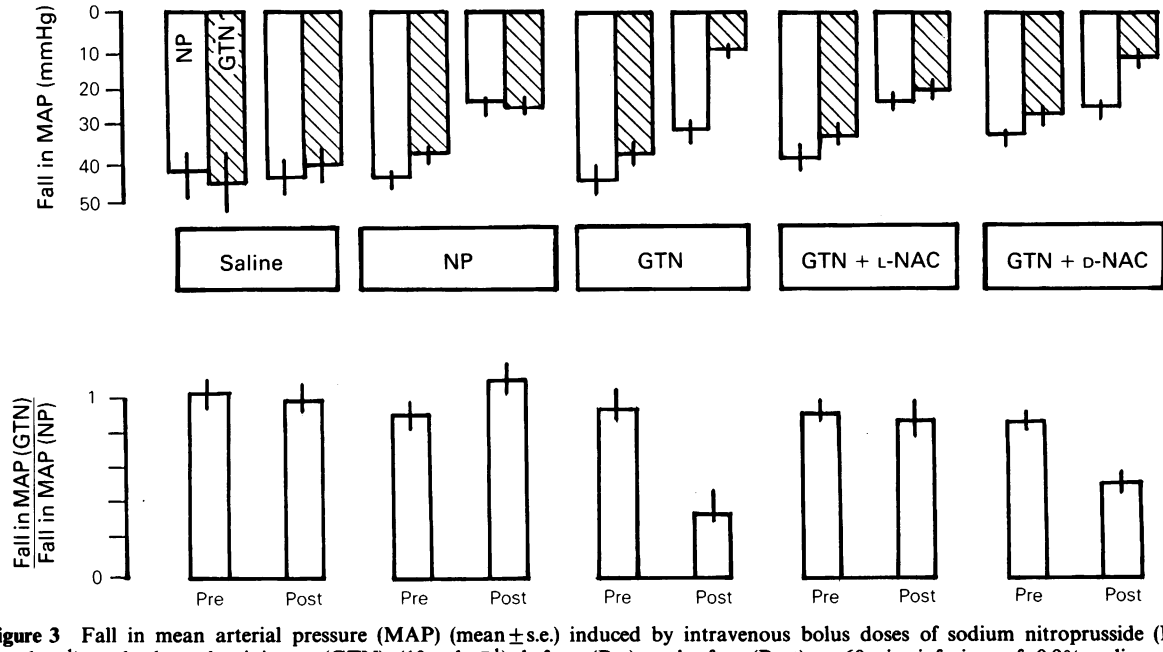

Figure 3 Fall in mean arterial pressure (MAP) (mean ± s.e.) induced by intravenous bolus doses of sodium nitroprusside (NP) (4 $\mu g\,kg^{-1}$) and glyceryl trinitrate (GTN) (10 $\mu g\,kg^{-1}$) before (Pre) and after (Post) a 60 min infusion of 0.9% saline, NP (20 $\mu g\,kg^{-1}\,min^{-1}$) or GTN (40 $\mu g\,kg^{-1}\,min^{-1}$). Shown in the bottom panel is the ratio of the fall in MAP induced by a bolus dose of GTN compared to that induced by a bolus dose of NP (GTN : NP ratio).

sulphydryl groups (Needleman & Johnson, 1973). More recently, Ignarro et al. (1981) showed that activation of partially purified soluble guanylate cyclase by organic nitrates, nitrites, nitroprusside and nitric oxide *in vitro* requires the addition of thiols; GTN differed from the other nitrocompounds tested in that cysteine was the only effective thiol donor. One interpretation of these data is that tolerance to GTN is secondary to a specific depletion of intracellular cysteine, rather than of the total thiol pool.

Intracellular cysteine may be replenished by L-NAC, which enters cells where it is de-acetylated to free L-cysteine. The intracellular concentration of L-cysteine is tightly controlled, largely by conversion to glutathione (Cooper, 1985) and levels above 150 μM cannot be achieved, even when isolated cells are incubated with high concentrations of L-NAC *in vitro* (Cotgreave et al., 1987). D-NAC will also readily enter cells, but it is not metabolised further (Sjodin et al., 1989). Assuming that D-NAC is distributed throughout total body water, then it can be estimated that the tissue concentration in our experiments would have been greater than one millimolar, thereby providing a much higher concentration of free cysteine-derived sulphydryl groups than the same dose of L-NAC. If the maintenance of an adequate concentration of free cysteine-derived sulphydryl groups for participation in a non-enzymatic reaction was the only requirement for the prevention of tolerance to GTN, then D-NAC should have been the more effective of the two enantiometers. In fact we found the exact opposite. Thus, our data are more compatible with an enzyme-dependent interaction between GTN and NAC and/or cysteine.

Whilst depletion of intracellular cysteine or other thiol compounds may represent the main cause of tolerance to organic nitrates, additional mechanisms of tolerance may exist, at least under experimental conditions. For example, some *in vitro* data suggest that tolerance may be induced to inorganic nitrocompounds (such as NP) as well as to GTN and other organic nitrates (Axelsson et al., 1982; Axelsson & Andersson, 1983; Keith et al., 1983; Molina et al., 1987). It has been suggested that the mechanism of this tolerance may involve desensitization of guanylate cyclase (Waldman et al., 1986; Schroder et al., 1988; Romanin & Kukowetz, 1989; Henry et al., 1989). However, any desensitization of guanylate cyclase observed in cell-free preparations *in vitro* may not accurately reflect the situation within intact cells (Mulsch et al., 1988). We found that infusions of either NP or GTN were associated with a moderate attenuation of the hypotensive effect of bolus doses of NP. Infusion of NP for 60 min caused a similar loss of potency of bolus doses of GTN. This second, nonspecific, cross-tolerance was not prevented by L-NAC, is therefore unlikely to be due to depletion of intracellular cysteine, and could be mediated at the level of guanylate cyclase. Further studies with guanylate cyclase-independent vasodilators are required to investigate this point further.

The original *in vivo* model of nitrate tolerance in the rat was developed by Needleman and involved repeated dosing over several days (Needleman, 1970). *In vitro* data suggested that tolerance to high concentrations of organic nitrates may occur within 1 h of exposure (Mulsch et al., 1988; Henry et al., 1989). We have been able to produce tolerance *in vivo* over a similarly short period by the use of a continuous intravenous infusion. The characteristics of the specific tolerance to GTN in our model, particularly its prevention by L-NAC, imply that the mechanisms involved are similar to those of clinical nitrate tolerance, and suggest that it is a suitable model for further *in vivo* studies.

The stereospecificity of the prevention of nitrate tolerance in this model by L-NAC suggests the involvement of an enzyme-dependent step in the interaction of GTN with NAC and/or cysteine. Additional work with other thiol donors is required to elucidate the interaction further.

References

ABDOLLAH, A., MOFFAT, J.A. & ARMSTRONG, P.W. (1987). *N*-acetylcysteine does not modify nitroglycerin-induced tolerance in canine vascular rings. *J. Cardiovasc. Pharmacol.*, **9**, 445–450.

AXELSSON, K.L., ANDERSSON, R.G.G. & WIKBERG, J.E.S. (1982). Vascular smooth muscle relaxation by nitro compounds: Reduced relaxation and cGMP elevation in tolerant vessels and reversal of tolerance by dithiothreitol. *Acta Pharmacol. Toxicol.*, **50**, 350–357.

AXELSSON, K.L. & ANDERSSON, R.G.G. (1983). Tolerance towards nitroglycerin, induced *in vivo*, is correlated to a reduced cGMP response and an alteration in cGMP turnover. *Eur. J. Pharmacol.*, **88**, 71–79.

COOPER, A.J.L. (1985). Biochemistry of sulfur-containing amino acids. *Ann. Rev. Biochem.*, **52**, 187–222.

COTGREAVE, I.A., SANDY, M.S., BERGGEN, M., MOLDEUS, P.W. & SMITH, M.T. (1987). *N*-acetylcysteine and glutathione-dependent protective effect of PZ51 (Ebselen) against diquat-induced cytotoxicity in isolated hepatocytes. *Biochem. Pharmacol.*, **36**, 2899–2904.

ELKAYAM, U., KULICK, D., McINTOSH, N., ROTH, A., HSUEH, W. & RAHIMTOOLA, S.H. (1987). Incidence of early tolerance to hemodynamic effects of continuous infusion of nitroglycerin in patients with coronary artery disease and heart failure. *Circulation*, **76**, 577–584.

GRUETTER, C.A. & LEMKE, S.M. (1985). Dissociation of cysteine and glutathione levels from nitroglycerin-induced relaxation. *Eur. J. Pharmacol.*, **111**, 85–95.

GRUETTER, C.A. & LEMKE, S.M. (1986). Effects of sulphydryl reagents on nitroglycerin-induced relaxation of bovine coronary artery. *Can. J. Physiol. Pharmacol.*, **64**, 1395–1401.

HENRY, P.J., HOROWITZ, J.D. & LOUIS, W.J. (1989). Nitroglycerin-induced tolerance affects multiple sites in the organic nitrate bioconversion cascade. *J. Pharmacol. Exp. Ther.*, **248**, 762–768.

IGNARRO, L.J., GRUETTER, C.A., HYMAN, A.L. & KADOWITZ, P.J. (1984). Molecular mechanisms of vasodilatation. In *Dopamine Receptor Agonists*. ed. Poste, G. & Crooke, S.T. pp. 259–288. New York: Plenum Publishing Corporation.

IGNARRO, L.J., LIPPTON, H., EDWARDS, J.C., BARICOS, W.H., HYMAN, A.L., KADOWITZ, P.J. & GRUETTER, C.A. (1981). Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: Evidence for the involvement of S-nitrosothiols as active intermediates. *J. Pharmacol. Exp. Ther.*, **218**, 739–749.

KEITH, R.A., BURKMAN, A.M., SOKOLOWSKI, T.D. & FERTEL, H. (1983). Vascular tolerance to nitroglycerin and cyclic GMP generation in rat aortic smooth muscle. *J. Pharmacol. Exp. Ther.*, **221**, 525–531.

MAY, D.C., POPMA, J.J., BLACK, W.H., SCHAEFER, S., LEE, H.R., LEVINE, B.D. & HILLIS, L.D. (1987). *In vivo* induction and reversal of nitroglycerin tolerance in human coronary arteries. *N. Engl. J. Med.*, **317**, 805–809.

MOLINA, C.R., ANDRESEN, J.W., RAPOPORT, R.M., WALDMAN, S. & MURAD, F. (1987). Effect of *in vivo* nitroglycerin therapy on endothelium-dependent and independent vascular relaxation and cyclic GMP accumulation in rat aorta. *J. Cardiovasc. Pharmacol.*, **10**, 371–378.

MULSCH, A., BUSSE, R. & BASSENGE, E. (1988). Desensitisation of guanylate cyclase in nitrate tolerance does not impair endothelium-dependent responses. *Eur. J. Pharmacol.*, **158**, 191–198.

NEEDLEMAN, P. (1970). Tolerance to the vascular effects of glyceryl trinitrate. *J. Pharmacol. Exp. Ther.*, **171**, 98–102.

NEEDLEMAN, P., JAKSCHIK, B. & JOHNSON, E.M. (1973). Sulphydryl requirement for relaxation of vascular smooth muscle. *J. Pharmacol. Exp. Ther.*, **187**, 324–331.

NEEDLEMAN, P. & JOHNSON, E.M. (1973). Mechanism of tolerance development to organic nitrates. *J. Pharmacol. Exp. Ther.*, **184**, 709–715.

PACKER, M., LEE, W.H., KESSLER, P.D., GOTTLIEB, S.S., MEDINA, N. & YUSHAK, M. (1987). Prevention and reversal of nitrate tolerance in patients with congestive heart failure. *N. Engl. J. Med.*, **317**, 799–804.

ROMANIN, C. & KUKOWETZ, W.R. (1989). Tolerance to nitroglycerin

is caused by reduced guanylate cyclase activation. *J. Mol. Cell. Cardiol.*, **21**, 41–48.

SCHRODER, H., LEITMAN, D.C., BENNETT, B.M., WALDMAN, S.A. & MURAD, F. (1988). Glyceryl trinitrate-induced desensitisation of guanylate cyclase in cultured rat lung fibroblasts. *J. Pharmacol. Exp. Ther.*, **245**, 413–418.

SHEFFNER, A.L., MEDLER, E.M., BAILEY, K.R., GALLO, D.G., MUELLER, A.J. & SARETT, H.P. (1966). Metabolic studies with acetylcysteine. *Biochem. Pharmacol.*, **15**, 1523–1535.

SJODIN, K., NILSSON, E., HALLBERG, A. & TUNEK, A. (1989). Metabolism of N-acetyl-L-cysteine. Some structural requirements for the deacetylation and consequences for oral availability. *Biochem. Pharmacol.*, **38**, 3981–3985.

WALDMAN, S.A., RAPOPORT, R.M., GINSBURG, R. & MURAD, F. (1986). Desensitisation to nitroglycerin in vascular smooth muscle from rat and human. *Biochem. Pharmacol.*, **35**, 3525–3531.

YEATES, R.A., LAUFEN, H. & LEITOLD, M. (1985). The reaction between organic nitrates and sulphydryl compounds. A possible model system for the activation of organic nitrates. *Molec. Pharmacol.*, **28**, 555–559.

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